EXINE STRUCTURE OF PANTOPORATE *CAMPANULA* (CAMPANULACEAE) SPECIES[1]

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ABSTRACT

The only species in Campanulaceae known to have pollen with pantoporate apertures are five species of *Campanula*: *C. americana*, *C. californica*, *C. exigua*, *C. griffinii*, and *C. sharsmithiae*. All other species examined of this large genus (ca. 300 species) have 3–4(–7) pores, spaced equidistant on the equator of the grain. The pantoporate species, especially the widely distributed annual or biennial herb *C. americana*, are further distinguished by elongate or well-developed columellae. The larger columellae of this species may be correlated with their lower density. Except for the similarities in pollen, *C. americana* does not appear closely related to the three slender annuals, *C. exigua*, *C. griffinii*, and *C. sharsmithiae*, each of which has a restricted distribution in California. *Campanula americana* also is not closely related to the pantoporate perennial, *C. californica*, or to the fourth California annual, *C. angustiflora*, which has 4–6-zonoporate pollen with a tectum and exine structure almost identical to *C. sharsmithiae*. The pantoporate grains of *C. californica*, with pores that are not always uniform in size or distribution, and the zonoporate grains of *C. angustiflora*, with unevenly distributed pores and elongate columellae, may represent transition stages between the two pollen types. In this study and previous ones, all species of Campanulaceae with well-developed or elongate columellae have been shown to have a high number (more than five) of apertures. Whether more apertures make the pollen wall more vulnerable to collapse and whether selective pressures have resulted in a more rigid wall by means of elongate columellae are debatable. The highly distinctive pollen-collecting hairs found in the Campanuloideae are illustrated and discussed.

In a systematic study of four closely related annual species of *Campanula*, all endemic to California, Morin (1980) established the existence of pantoporate (pores distributed over the entire surface) pollen in three, *C. exigua* Rattan, *C. griffinii* Morin, and *C. sharsmithiae* Morin, and 4–6-zonoporate pollen in *C. angustiflora* Eastwood. Later, Morin (unpublished) found the pantoporate aperture type in the weakly perennial *C. californica* (Kell. Heller). Prior to her studies only one other species in Campanulaceae, the robust annual or biennial *Campanula americana* L., native to eastern and central North America, was known to have a pantoporate aperture type (Avetisian, 1967; Dunbar, 1973a, b, 1975a, b, 1981, 1984; Shetler, 1982; for life history of *C. americana* see Shetler, 1958, and Baskin & Baskin, 1984). The unusual and very limited occurrence of pantoporate pollen in Campanulaceae prompted this investigation of the exine structure of *Campanula americana* and the California pantoporate species.

Exines subjected to partial degradation by plasma-ashing suggest that the fundamental tectum/sculpture in the subfamily Campanuloideae are rod-like or threadlike structures.

Although not specifically studied here, the pollination biology of *Campanula*, the species of which show pronounced protandry and adaptations to insects, is characterized by a unique and still largely unexplained mechanism. While the flower is still in bud, anthers dehisce and deposit the pollen on the pollen-collecting hairs of the upper style. As anthesis proceeds, both pollen and the unicellular hairs gradually disappear, the former owing to pollinator activity, the latter to retraction/invagination into their expanded bases. The fact that in some cases the retracted hair is observed "to trap" a pollen grain has prompted speculation that the invagination is an adaptation for autogamy (for review, see Shetler, 1979). This does not seem to be the case (Shetler, 1982; Lloyd & Yates, 1982), however; the possible functions of the collecting hairs are presented in the discussion section.

MATERIALS AND METHODS

For examination of pollen, anthers were removed from herbarium material and routinely acetolyzed (Erdtman, 1966) for all preparations: light microscopy (LM), scanning electron microscopy

copy (SEM), transmission electron microscopy (TEM), and plasma-ashing.

For SEM, pollen was coated with carbon, then gold-palladium, and examined and photographed with a Cambridge 250 Stereoscan Electron Microscope or a Hitachi 570 SEM.

For TEM, pollen was incorporated into agar, fixed with osmium tetroxide, stained with uranyl acetate, and embedded in Spurrs resin or L. R. White. After sectioning, the pollen was stained in lead citrate and examined in a JEOL 100, 1200EX, or a Philips 200 transmission electron microscope.

For LM, pollen was mounted in glycerin jelly and sealed with paraffin. Measurements (see Table 1) are based on 20 grains in LM.

For examination of collecting hairs, styles were dissected from herbarium flowers and attached to specimen stubs, coated with gold-palladium, and examined and photographed in a Cambridge 250 or an S410 scanning electron microscope. The collections examined are listed in Table 1.

In plasma-ashing an electrical field changes diatomic oxygen into excited oxygen ions to form a highly reactive plasma that causes low temperature combustion of organic molecules. The most common use of plasma-ashing is for removal of organic matter from inorganic structures, e.g., tissue from sponge spicules. For this application, end-point detectors can determine precisely when the organic layers have been removed. In completely organic samples such as exines, however, the only control (available to us) is duration of ashing. Based on an earlier study of pollen from 33 species representing 10 families (Nowicke et al., 1986), the first hour of ashing produces the most profound effect—a reduction in the sculpturing components resulting in skeletonlike exines. For greater detail, see Nowicke et al. (1986).

All LM slides and EM micrographs are deposited at the Palynological Laboratory, National Museum of Natural History, Smithsonian Institution.

RESULTS

In the course of the entire Campanulaceae study, the pollen of 58 collections representing 41 species from nine genera was examined in LM and SEM, and 21 of the 41 were examined in TEM. Twenty-eight species of *Campanula* were examined in LM and SEM, 14 of which were also examined in TEM. For the most part only the species that are cited in the text and/or illustrated are given in Table 1.

All 18 species of *Campanula* cited in Table 1 have oblate-spheroidal or spheroidal pollen with porate apertures and a tectum that consists of spinules and irregularly oriented threadlike or rodlike structures (Figs. 1–41, 54, 55, 57, 58). For porate Campanulaceae, Dunbar (1984: 1) classified surface sculpture types into the following categories: ridges; ridges, with top end bent upwards; fingerlike structures; protrusions; irregular ridges; and reticulum in low relief. With the exception of the last category, applicable only to *Campanula americana* in our study, we believe that these closely related categories are minor variations in the orientation of the rods or threads. Although the term rod, with its implication of a straight or erect linear structure, may be more fitting for some tecta than others, e.g., Figure 41 versus Figures 55 or 58, we will use rod in the remainder of the text to describe the ropelike strands making up the surface of the tectum, often intertwined in spaghettilike fashion. These rods can vary in their size, degree of distinction (e.g., Figs. 15, 31 vs. 9, 41), extent of projection (e.g., Fig. 35 vs. 38, 41), and orientation (e.g., mostly horizontal as in Fig. 35 or with rods more erect/vertical as in Figs. 17, 23, and 46).

In SEM, the relationship between the rods and spinules seems clear—the free tips of rods appear to coalesce to form spinules. In every *Campanula* species examined in SEM, the base of at least some spinules is continuous with three or more rods. This condition is illustrated best in the plasma-ashed samples, Figures 56 and 59, but almost as well in Figures 8, 29, 38, 41, and 58. In the past (Dunbar, 1973a; Morin, 1980), the spinules have been considered as basally rooted or divided, but we now think that the spinules are the result of free tips of rods coalescing and not of rods formed by the basal subdivision of spinules (see discussion below).

In thin section, all species examined have stratified exines with an endexine, foot layer, columellae, and tectum, but these components are variously developed.

Based on the above characteristics the pollen of *Campanula* is a recognizable type: porate with a tectum consisting of spinules and variously oriented rods. But the distribution of the pores defines two distinctive subtypes with very unequal frequencies. Five species are pantoporate (Figs. 1, 7, 13, 21, and 24), with as few as six apertures and as many as 18. All remaining campanulas have three, sometimes four, or more rarely five or six (seven in a few grains of *C. rotundifolia*) pores placed equidistant on the equator of the grain (some grains of *C. angustiflora* excepted).

Our TEM investigation revealed subtle differences in the structure of the exine: in the panto-

TABLE 1. Species examined, voucher data, pollen data, and figure numbers.

Species	Collection[1]	Location	Figure(s)	Grain size[2]	Pore size	Number of pores
Campanula alpina Jacq.	*Bujorean & Nyarady s.n.* 7/1/23	Romania	28–30	P(28) 30 (32) × E(30) 32 (35)	4–5.2	3
C. americana L.	*Demaske 2128*	Wisconsin		(36) 38 (40)	5.2–6.5	12–15
	Shimek s.n. 8/15/27	Iowa	3–6	(35) 38 (40)	5.2–6.5	10–13
	Soper & Dale 4081	Canada	1, 2	(35) 38 (39)	5.2–6.5	10–13
	Hartley 1472	Wisconsin	60, 62–69			
	Ward s.n. 4/7/1878	Maryland	61			
C. angustiflora Eastwood	*Constance et al. 3045*	California	16–20	P(29) 31 (32) × E(31) 34 (36)	4–6.5	(4–)6
C. barbata L.	*Hermann 19483*	France	33–35	P(29) 32 (34) × E(30) 31 (34)	5.2–6.5	3
C. baumgartenii J. Becker	*Hall 3125*	Germany	57–59	P(27) 29 (31) × E(30) 31 (43)	5.2	3–4
C. californica (Kell.) Heller	*McMurphy 22*	California	24–27	(39) 40 (45)	2.6–4	7–11
C. divaricata Michx.	*Allard 2116*	Virginia		P(30) 32 (35) × E(32) 35 (36)	5.2–6.5	3
	Duncan 11925	South Carolina		P(29) 31 (34) × E(32) 34 (36)	5.2	3
C. exigua Rattan	*Sharsmith & Sharsmith 3358*	California	7, 8, 10–12	(36) 40 (42)	5.2	12–14
	Morin 297 (MO)	California	9	(34) 35 (38)	4–5.2	12–15
C. glomerata L.	*Collins & Fernald s.n.* 1904	Canada		P(29) 30 (34) × E(31) 33 (38)	4.5–6.5	(2) 3 (4)
C. griffinii Morin	*Griffin 4120* (MO)	California	13, 14	(30) 31 (34)	5.2–6.5	6–9
	Howell 21813	California	15	(30) 33 (35)	4–5.2	7–12
C. hagielia Boiss.	*Rechinger 3678*	Greece	31, 32	P(21) 23 (25) × E(22) 25 (26)	3.9–5.2	3 (4)
C. lasiocarpa Cham.	*Hulten s.n.* 7/15/61	Alaska		P(30) 32 (35) × E(31) 33 (35)	5.2–6.5	3–4
C. macrorhiza Gay ex A. DC.	*Wilczek & Dutoit* 21-VII-30	France		P(27) 29 (30) × E(29) 31 (33)	4–5.2	(2) 3 (4)
C. medium L.	*Thomas 10926*	Montana	54–56	P(34) 37 (39) × E(36) 39 (40)		3
C. rhomboidalis L.	*Maillefer 37599*	Switzerland	36–38	P(26) 29 (31) × E(28) 29 (34)	4–5.2	3 (4)
C. rotundifolia L.	*Charpin et al. s.n.* 7/15/69	Italy	39	P(32) 37 (40) × E(34) 36 (41)	5.2–6.5	3–4
	Terrell & Brown 3858	Colorado	40, 41	P(30) 33 (38) × E(34) 36 (41)	5.2–6.5	4 (5)
C. sharsmithiae Morin	*Morin 301* (MO)	California	21–23	(35) 38 (40)	4–5.2	12–18
C. sibirica L.	*Csato 2977*	Romania		P(29) 32 (34) × E(31) 33 (36)	5.6–7.1	(2) 3 (4)
Codonopsis bulleyana Forrest ex Diels	*Rock 5444*	China	51–53	P(41) 44 (48) × E(41) 46 (49)	17–36	6–8
C. convolvulacea Kurs	*Rock 6603*	China	42–44	P(38) 42 (48) × E(43) 46 (51)	7.8–13	6
Githopsis pulchella Vatke	*Constance s.n.* 5/19/51	California	45–47	P(36) 40 (45) × E(39) 43 (47)	5.8–7.8	6
Wahlenbergia linarioides DC.	*Schwarz 10877*	Argentina	48–50	P(25) 28 (30) × E(29) 31 (32)	4.5–5.2	3 (4)

[1] All US unless otherwise indicated.

[2] Does not include spinules; P = polar dimension, E = equatorial dimension; numbers in ( ) indicate range.

porate type the prominent or elongate columellae project through the tectum to form the core of most spinules, e.g., Figures 3–5, 10, 11, 19, 20, 27, whereas in the zonoporate type the columellae are reduced (e.g., Figs. 30, 32, and 37) and the spinules are not formed by columellae (see above discussion of SEM data). The zonoporate type usually has a well-developed foot layer (Figs. 32, 34, 37).

A complete description of each pollen type is given below; for pollen size, the range of the mean (Table 1) is given.

PANTOPORATE POLLEN TYPE—FIGURES 1–15, 21–27

Pollen spheroidal, 31–40 μm diam., with 6–18 pores, the pores 4–6.5 μm diam., the tectum consisting of rods and spinules, or microreticulate and spinulate; exine structure consisting of lamellate endexine prominently thickened at the pores; the foot layer mostly thin or sometimes equal in thickness to the nonapertural endexine; the columellae elongate, prominent, at least some of which project through the tectum to form the core of most spinules; the tectum incomplete or nearly complete (*C. griffinii*). Five species: *Campanula americana, C. californica, C. exigua, C. griffinii,* and *C. sharsmithiae.*

Campanula americana (Figs. 1–6) is 12–15-pantoporate with a microreticulate tectum in which horizontal rods are muri or vice versa. No other species of *Campanula* examined in this study or illustrated in others (Dunbar, 1973a, b, 1975a, 1984; Morin, 1980) has a microreticulate tectum or such massive columellae. The rods/muri and spinules are more evident in some grains than in others. In thin section (Figs. 3–5), the exine of *C. americana* consists of a lamellate endexine present throughout the grain but greatly thickened near the pores and with a thin foot layer. Comparison of radial sections of *C. americana* (Fig. 3) with those of other pantoporate species, e.g., *C. exigua* (Fig. 11), *C. sharsmithiae* (Fig. 22), and *C. californica* (Fig. 27), as well as zonoporate species, e.g., *C. alpina* (Fig. 30) and *C. hagiela* (Fig. 32), emphasizes the unusual development of the columellae in *C. americana.* Larger columellae may be a compensation for, or correlation with, fewer columellae. The density of columellae in the tangential section of *C. americana* (Fig. 4) is much lower than that in *C. exigua* (Fig. 10): when a grid one centimeter square was variously placed over the area occupied by columellae, the highest number of columellae for *C. americana* was 10, while that for *C. exigua* was 30.

The pollen of the remaining pantoporate species, *C. californica* (Figs. 24–27), *C. exigua* (Figs. 7–12), *C. sharsmithiae* (Figs. 21–23), and *C. griffinii* (Figs. 13–15), was examined in LM and SEM. The first three species were also examined in TEM, as was the zooporate annual, *C. angustiflora.*

The weakly perennial *Campanula californica* (Figs. 24–27) has the elongate columellae associated with the pantoporate type, but the distribution and size of the pores are not uniform. In LM there are numerous grains that, in optical section, have four to six pores on the "equator"; each hemisphere (top and bottom focus) may have only one pore, or one hemisphere may have none at all; in more rare cases neither hemisphere has a pore; very frequently one of the pores is distinctly smaller. The elongate spinules, obvious even in LM, would distinguish the pollen of *C. californica* from all remaining pantoporate types. These observations suggest that the pollen of *C. californica* is intermediate between the two pollen types, but closer to the pantoporate than to the zonoporate.

Campanula exigua (Figs. 7–12) is 12–15-pantoporate with well-defined, short, sometimes erect rods (Figs. 8, 9). In thin section the electron densities of the foot layer and endexine are similar (Fig. 11), making it difficult to characterize them individually, but together they form a well-defined unit that becomes much thicker near the pores. The columellae, although not as large as in *C. americana,* are the predominant layer in mesoporal (nonapertural) areas, and most appear to project through the irregular tectum to form the core of the spinules. Their elongation is underscored in tangential section (Fig. 11) by the proportion of the total area they occupy. In both collections examined, the rods (Figs. 8, 9) are distinct from each other and are short, and some appear vertical.

One collection of *C. griffinii, Griffin 4120* (Figs. 13, 14), was 7–9-pantoporate with a typically campanulaceous tectum; the other, *Howell 21813* (Fig. 15), was 8–11-pantoporate with more poorly defined tectal rods.

Campanula sharsmithiae (Figs. 21–23) is 12–18-pantoporate with a tectum that appears to consist of irregularly defined elements as well as rods. In thin section (Fig. 22) the exine of this species consists of a thick, mostly lamellate endexine that is slightly less electron dense than the thin foot layer and well-developed columellae, some of which project through the tectum to form spinules. The exine structures in *C. exigua* and *C. sharsmithiae* are similar.

In contrast to *Campanula americana, C. cal-*
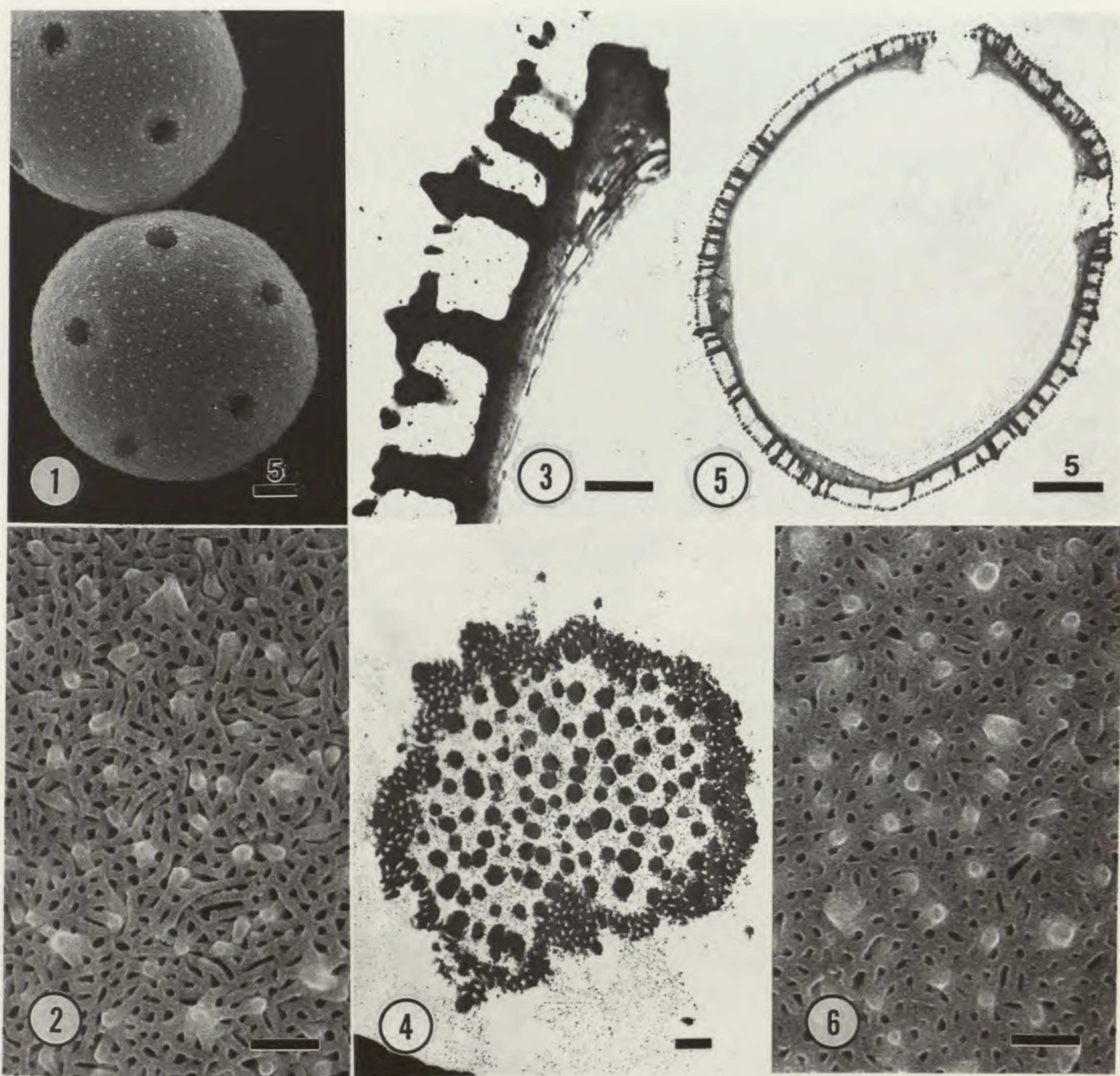
FIGURES 1–6. SEM and TEM of *Campanula americana* pollen.—1. SEM of two pantoporate grains.—2. SEM of tectum.—3. TEM of radial section including part of pore. Note massive columellae, some of which project through the tectum, and thickened, lamellate endexine nearer the pore.—4. TEM of tangential section. Perforated tectum (outer ring) agrees with that portrayed in SEM, Figures 2 and 6.—5. TEM of mostly radial section through whole grain.—6. SEM of tectum. Scale bars = 1 μm unless otherwise indicated.

ifornica, C. exigua, C. griffinii, and *C. sharsmithiae,* all other campanulas, to our knowledge, have pollen that is 3–4(–7)-zonoporate.

ZONOPORATE POLLEN TYPE—
FIGURES 16–20, 28–41, 54, 55, 57, 58

Pollen mostly oblate-spheroidal, Polar diameter (23–)29–37 μm × Equatorial diameter (25–)31–39 μm, 3–4 pores (rarely 7, *C. rotundifolia*), the pores circular or slightly elongated polarly (= lolongate), the longest dimension 4.0–6.5 μm, the tectum consisting of irregularly oriented rods, frequently with a free tip, some of which coalesce to

form spinules; exine structure consists of a lamellate endexine that becomes much thicker near the pores; a well-developed foot layer that is thicker than the nonapertural Cendexine; reduced or short columellae; and a complete or almost complete tectum, with small to large spinules that are not continuous with columellae.

The pollen of seven zonoporate species, *C. alpina* Jacq. (Figs. 28–30), *C. angustiflora* (Figs. 16–20), *C. barbata* L. (Figs. 33–35), *C. baumgartenii* J. Becker (Figs. 57, 58), *C. hagiela* Boiss. (Figs. 31, 32), *C. rhomboidalis* L. (Figs. 36–38), and *C. rotundifolia* L. (Figs. 39–41), is illustrated here in LM, SEM, and TEM. With the exception
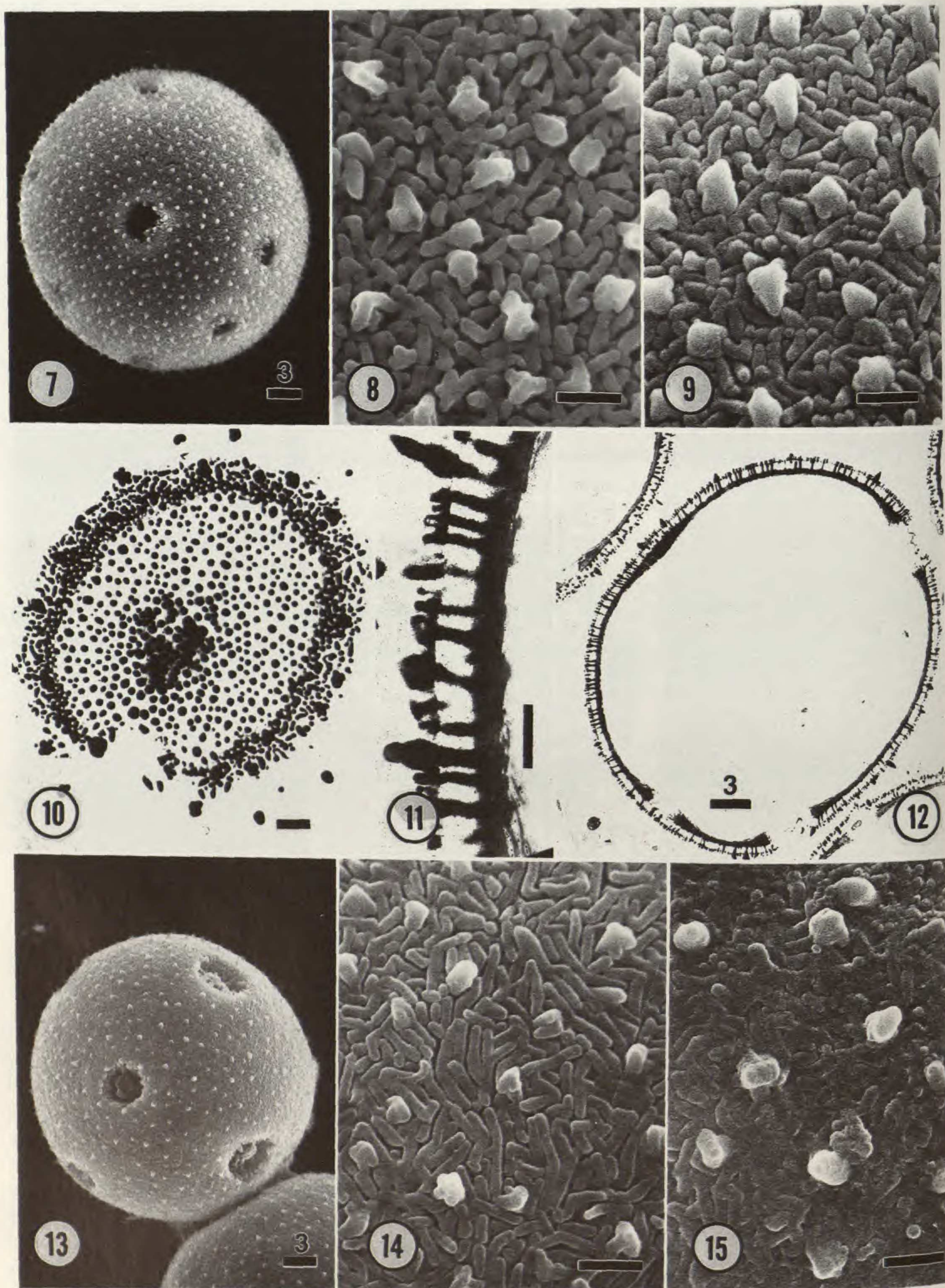
FIGURES 7–15. SEM and TEM of *Campanula* pollen. 7-12. *C. exigua.*—7. SEM of pantoporate grain.—8. SEM of tectum.—9. SEM of tectum from another collection (see Table 1).—10. TEM of tangential section. Note tectal perforations and the large area occupied by columellae cut at right angle to their long axes.—11. TEM of radial section. Note prominent columellae, some of which project through tectum.—12. TEM of section of whole grain. Note buildup of endexine near apertures. 13–15. *C. griffinii.*—13. SEM of whole grain. Note larger and fewer apertures than in the remaining pantoporate species.—14. SEM of tectum.—15. SEM of tectum from another collection (see Table 1). Scale bars = 1 μm unless otherwise indicated.
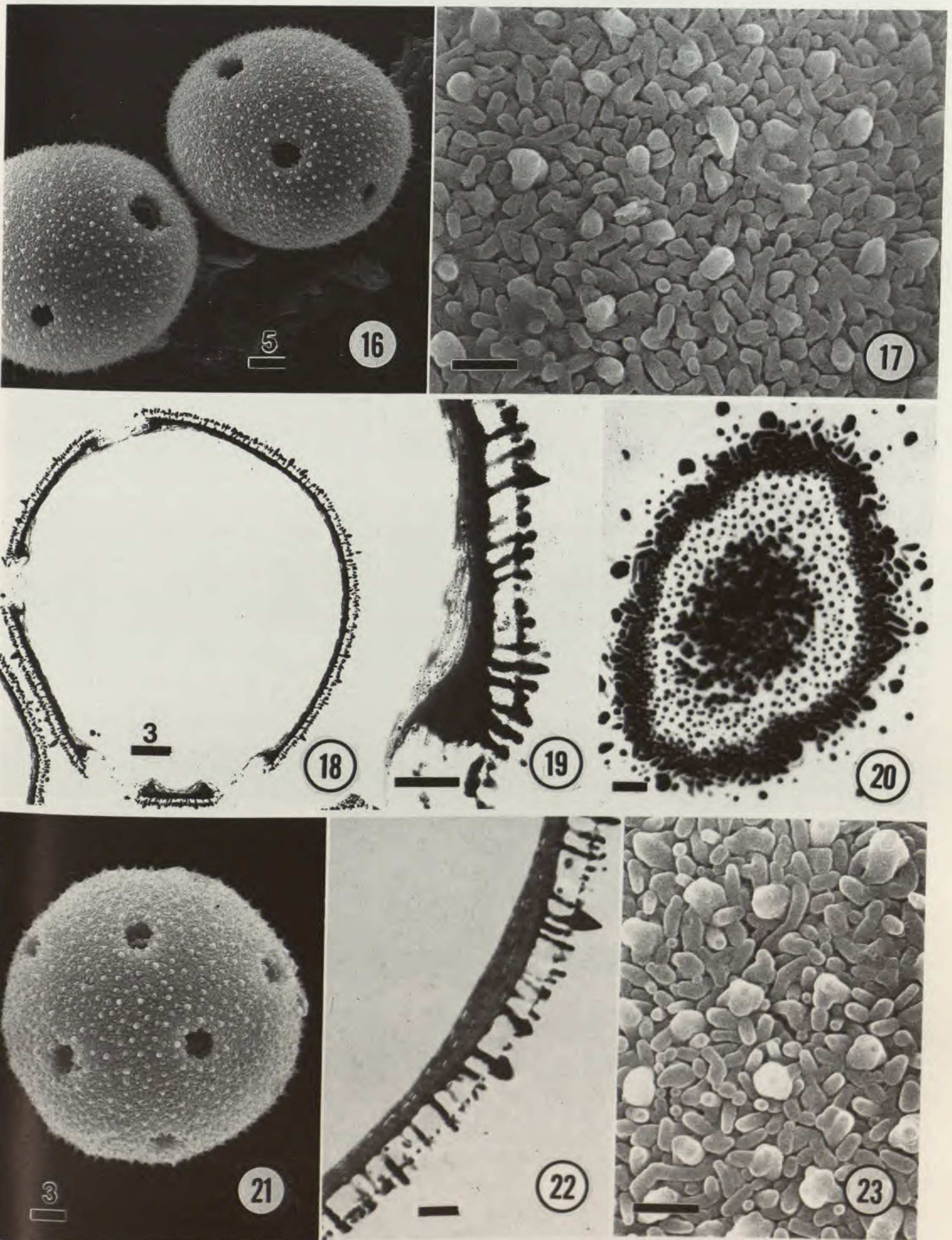
FIGURES 16–23. SEM and TEM of *Campanula* pollen. 16–20. *C. angustiflora.*—16. SEM of zonoporate grains. Note irregularity of pore distribution and size.—17. SEM of tectum.—18. TEM of section of whole grain. The fact that the four visible apertures are similar in size indicates that the section is close to being parallel with the actual equator of the grain. If there are apertures on the right side they are well above or below the equator.—19. TEM of radial section including part of pore. Note that some columellae project through the tectum.—20. TEM of tangential section. Compare columellae size and area occupied with Figures 4 and 10. 21–23. *C. sharsmithiae.*—21. SEM of pantoporate grains.—22. TEM of radial section. The thickened and lamellate endexine indicates proximity to an aperture.—23. SEM of tectum. Compare with tectum of *C. angustiflora*, Figure 17. Scale bars = 1 μm unless otherwise indicated.
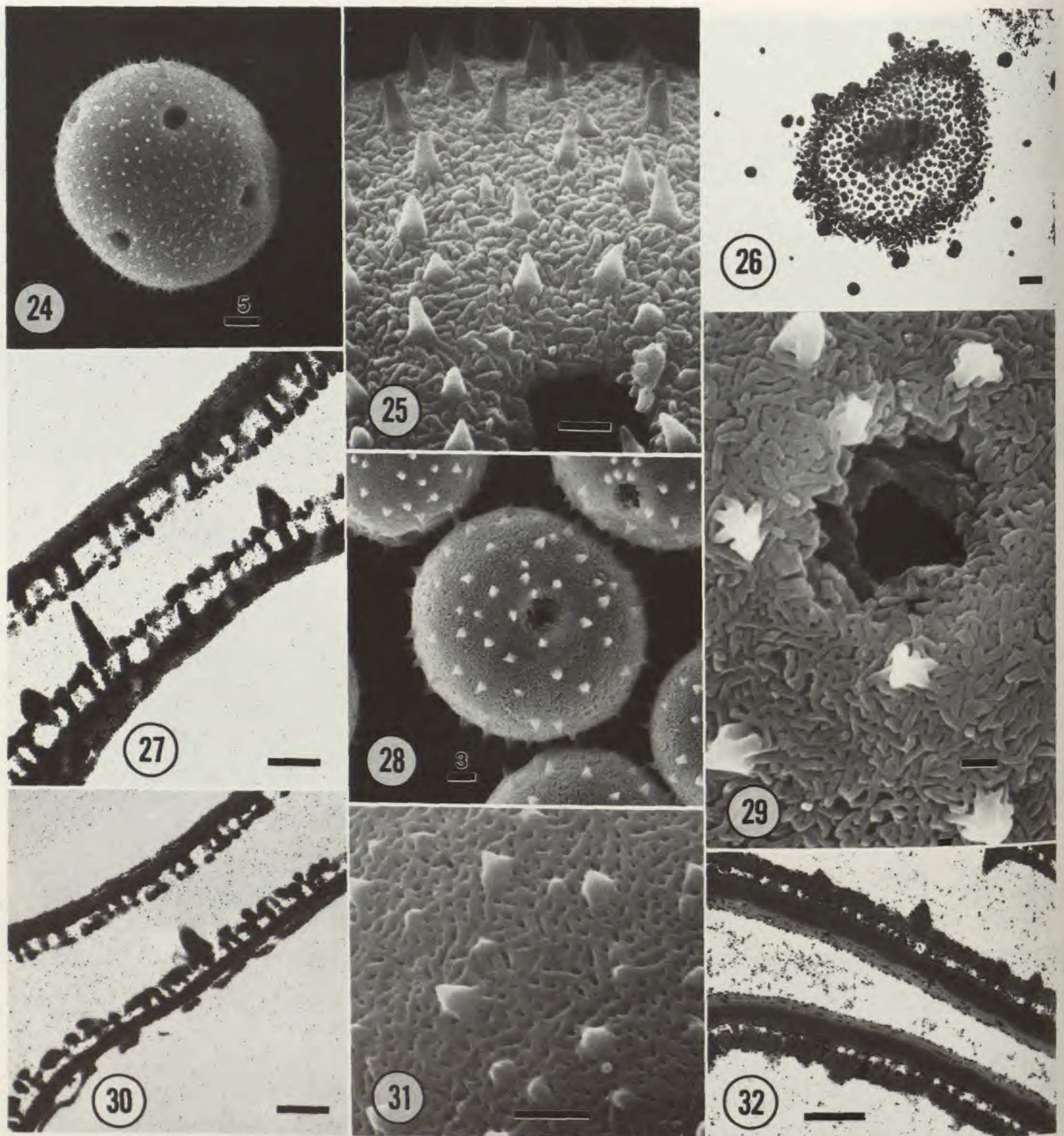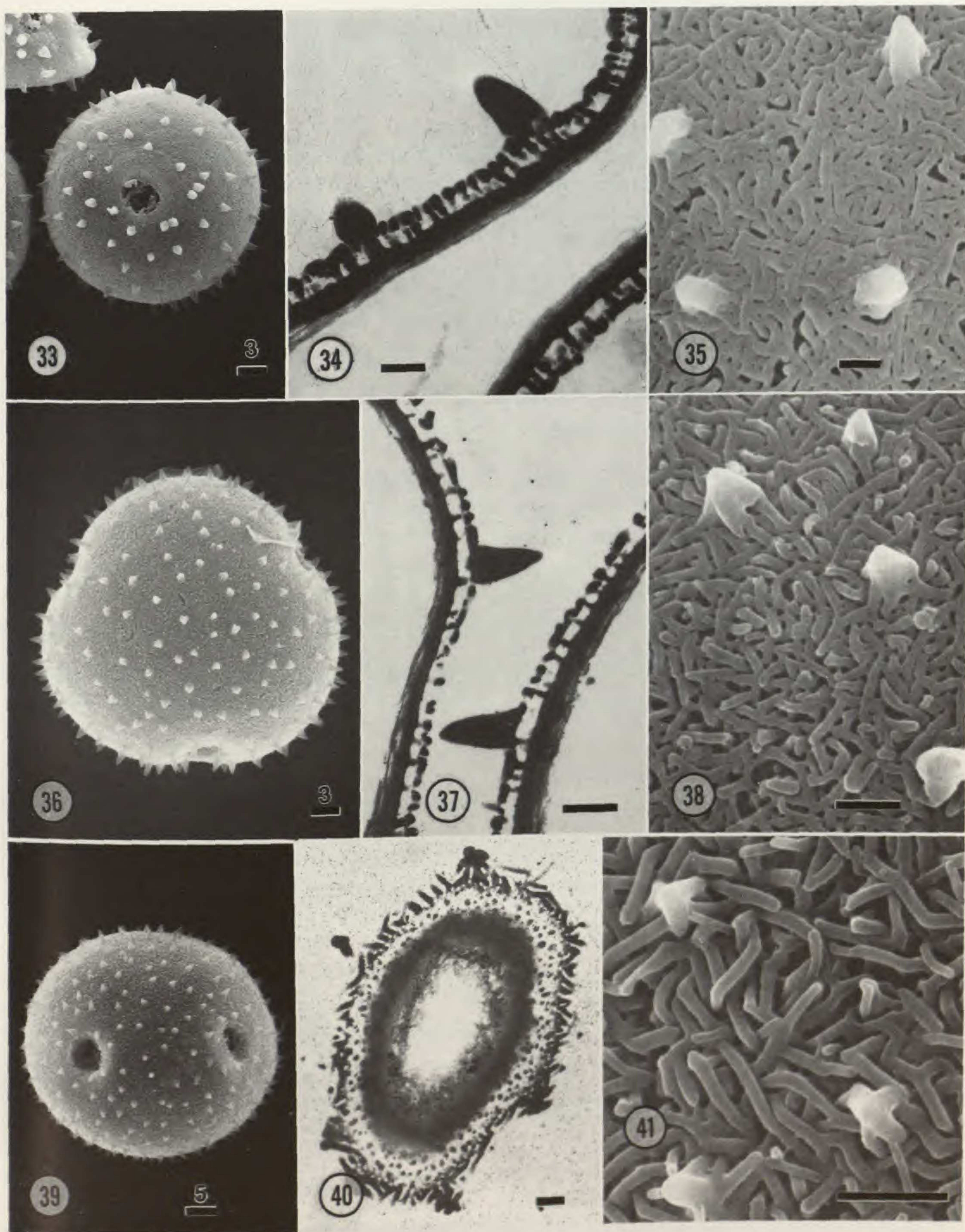
FIGURES 24–32. SEM and TEM of *Campanula* pollen. 24–27. *C. californica.*—24. SEM of pantoporate grain. Note long spines.—25. SEM of tectum with part of pore.—26. TEM of tangential section. Compare the diameter of these columellae with those in Figure 10 of *C. exigua* and in Figure 37 of *C. rotundifolia.*—27. TEM of slightly oblique radial sections of two grains. Note elongate columellae and the direct connection with spinules. 28–30. *C. alpina.*—28. SEM of zonoporate grain in equatorial view.—29. SEM of tectum with pore. Note direct relationship of spines with rods.—30. TEM of oblique sections of two grains. 31, 32. *C. hagiela.*—31. SEM of tectum. Note small irregular-sized spinules and lack of distinction of rods.—32. TEM of radial section of collapsed grain including two walls. Note well-developed foot layer, small columellae, and almost continuous tectum. Scale bars = 1 μm unless otherwise indicated.

of *C. angustiflora,* discussed separately below, the exines of the above species are similar: irregularly oriented rods that are distinct, e.g., Figure 41, or barely visible, e.g., Figure 31, and poorly developed or short columellae with the foot layer/endexine the predominant component (Figs. 30, 32, 34, 37, 40).

Campanula rhomboidalis (Figs. 36–38) has pollen with three pores on the equator of the grain and a tectum (Fig. 38) that is representative of the genus. In TEM (Fig. 37) the most prominent unit is the foot layer/endexine, while the columellae are very short.

Of the zooporate species examined in TEM,

Figures 33–41.   SEM and TEM of *Campanula* pollen. 33–35. *C. barbata.*—33. SEM of zonoporate grain.— 34. TEM of radial section.—35. SEM of tectum. 36–38. *C. rhomboidalis.*—36. SEM of polar view of 3-porate grain.—37. TEM of slightly oblique radial sections of two grains.—38. SEM of tectum. 39–41. *C. rotundifolia.*— 39. SEM of equatorial view of (probably) 4-zonoporate grain.—40. TEM of tangential section. The most prominent component is the foot layer.—41. SEM of tectum. Scale bars = 1 μm unless otherwise indicated.

Campanula barbata (Fig. 34) has the most well-defined, albeit short, columellae.

Although the pollen of *Campanula angustiflora* (Figs. 16–20) is zonoporate, the higher number of pores (4–6) and their somewhat irregular distribution—not necessarily equidistant from each other and not all on the equator—distinguishes this pollen from many zonoporate types. Moreover, the tectum and exine structure link this annual to the pantoporate ones. Some grains of *C. angustiflora* (Fig. 17) have a tectum that is almost indistinguishable from some grains of *C. sharsmithiae* (Fig. 23)—both have irregularly shaped elements in addition to rods. Most likely these elements are derivatives of the horizontal rods; perhaps they are only the free tips. Clearly, the exine structure is more similar to that of the pantoporate species than to the zonoporate species: elongate columellae in *C. angustiflora* (Fig. 19) project through the tectum to form spinules like the pantoporate taxa (Fig. 22).

For purposes of comparison, species of three other genera of Campanulaceae are illustrated here: *Codonopsis convolvulacea* Kurs (Figs. 42–44), *Githopsis pulchella* Vatke (Figs. 45–47), and *Wahlenbergia linarioides* DC. (Figs. 48–50).

Codonopsis convolvulacea (Figs. 42–44) is 7-zonocolpate with short colpi and a tectum with densely spaced rods and large spinules. In TEM (Fig. 44), however, it is distinct from most Campanulaceae: a well-defined endexine, no foot layer, and columellae that appear to terminate abruptly, with an outer irregular layer seemingly composed of very short columellae connected to the rods. Some inner columellae narrow at the interface and then become expanded again in the outer layer of columellae. None of the grains examined in TEM had a foot-layer, and the columellae extended as such to the endexine. *Codonopsis bulleyana* Forrest ex Diels, Figures 51 and 52, also examined in TEM but not illustrated here, has a very generalized exine structure with a definite foot layer and simple columellae (Nowicke, unpublished data).

Githopsis pulchella (Figs. 45–47) is 6–7(–8)-zonoporate, as in other species of the genus (Morin, 1983), with a tectum remarkably similar to *Campanula angustiflora* (Fig. 17) and *C. sharsmithiae* (Fig. 23) in which the rods appear more erect. The fact that the radial section in Figure 47 is somewhat oblique has enhanced the thickness of the foot layer/endexine and obscured the continuity of most columellae as spinules. Other sections (Nowicke, unpublished) show most, if not all, columellae projecting through the tectum. Note also the resemblance of the inner surface of the tectum in the

lower grain in Figure 47 to *Codonopsis convolvulaceae* (Fig. 44).

The pollen of *Wahlenbergia linarioides* (Figs. 48–50) is 3-zonoporate with a typically campanulaceous tectum and exine structure.

As part of an earlier study (Nowicke et al., 1986) of substructure of two distinct pollen types (a compound layer of striae [= lirae] and a derived triangular array) that occurred together in a number of families, exines of Berberidaceae, Cistaceae, Euphorbiaceae, and Geraniaceae were partially degraded by plasma-ashing (see Materials and Methods). To better judge the effect of plasma-ashing on these two pollen types, additional exines, including species of Campanulaceae, were ashed and examined in SEM. Control (nonashed) and ashed exines of *Codonopsis bulleyana* (Figs. 51–53), *Campanula medium* (Figs. 54–56), and *C. baumgarteni* (Figs. 57–59) are illustrated in high-magnification SEMs. In the two *Campanula* species, plasma-ashing (Figs. 56, 59) clearly shows the rod-like substructure of the spinules as well as the tectum. In *Codonopsis bulleyana*, however, there are no spinules, and in the control there is no evidence of rods (Figs. 51, 52), unlike in a second species, *Codonopsis convolvulaceae* (Figs. 39–41). After plasma-ashing for 60 minutes, a network (Fig. 53), not unlike the rods in typical campanulaceous tecta, is evident. The small protuberances could be interpreted as vestiges of the free tip of many rods. Thus the tectum of *C. bulleyana* (Fig. 52) may represent a reduced stage in which the identity of the rods (as well as spinules) has, for all intents and purposes, been lost, or it may represent a primitive state in which these elements have not yet become differentiated.

DISCUSSION

For the most part our results agree with those of Dunbar (1984), who characterized the pollen of *Campanula* as having bacula that "are generally short and stubble-like except in one species where they are high." The exception to which she referred is *C. americana*. Dunbar, although aware of the existence of the pantoporate California species, had no opportunity (apparently) to examine them in thin section.

The relationship between *Campanula americana* and the four pantoporate California species is perplexing. Although the "microperforate" tectum of *C. americana* distinguishes it from the California species, all five species share two very restricted, at least in *Campanula*, pollen characters—pantoporate aperture type and elongate col-
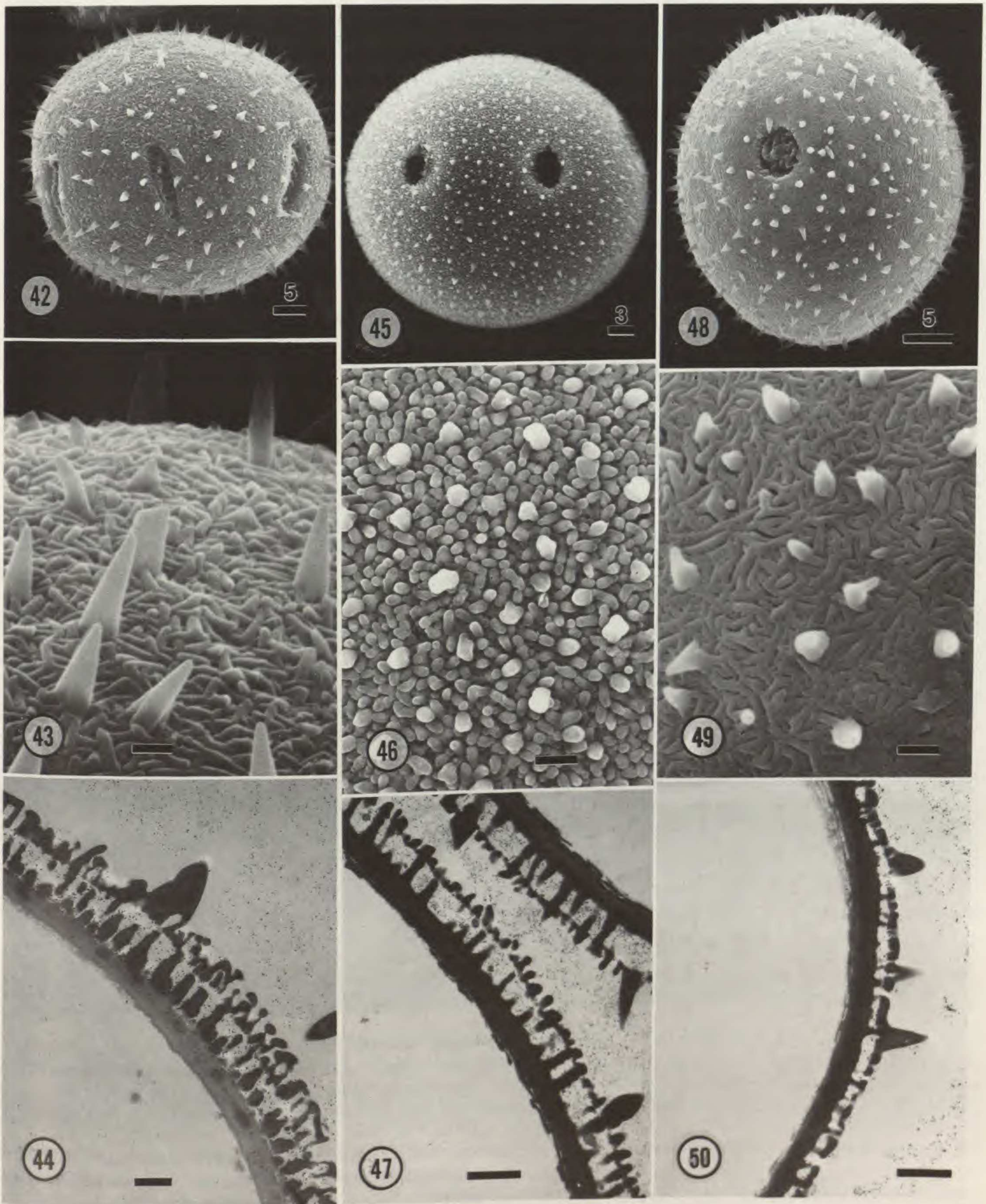
FIGURES 42–50. SEM and TEM of Campanulaceae pollen. 42–44. *Codonopsis convolvulacea.*—42. SEM of equatorial view of 7–8-brevicolpate grain.—43. SEM of tectum showing elongate spines and distinct, interwoven rods.—44. TEM of slightly oblique radial section. Note unusual structure of ektexine: the apparent absence of a foot layer, the truncated columellae, and the irregular tectum. Compare this species with *Codonopsis bulleyana* (Figs. 51, 52). 45–47. *Githopsis pulchella.*—45. SEM of slightly oblique view of 5–7-zonoporate grain.—46. SEM of tectum. Compare with *C. angustiflora* (Fig. 17) and *C. sharsmithiae* (Fig. 23).—47. TEM of slightly oblique radial sections of two grains. The electron density of the endexine is so similar to that of the foot layer that their common boundary is difficult to discern, but the endexine is thicker; note that some columellae project through tectum to form core of some spinules. 48–50. *Wahlenbergia linarioides.* —48. SEM of equatorial view of 3-zonoporate grain.—49. SEM of tectum.—50. TEM of radial section. Note thick foot layer, diminutive columellae, and spinules that are not extensions of columellae. Scale bars = 1 μm unless otherwise indicated.
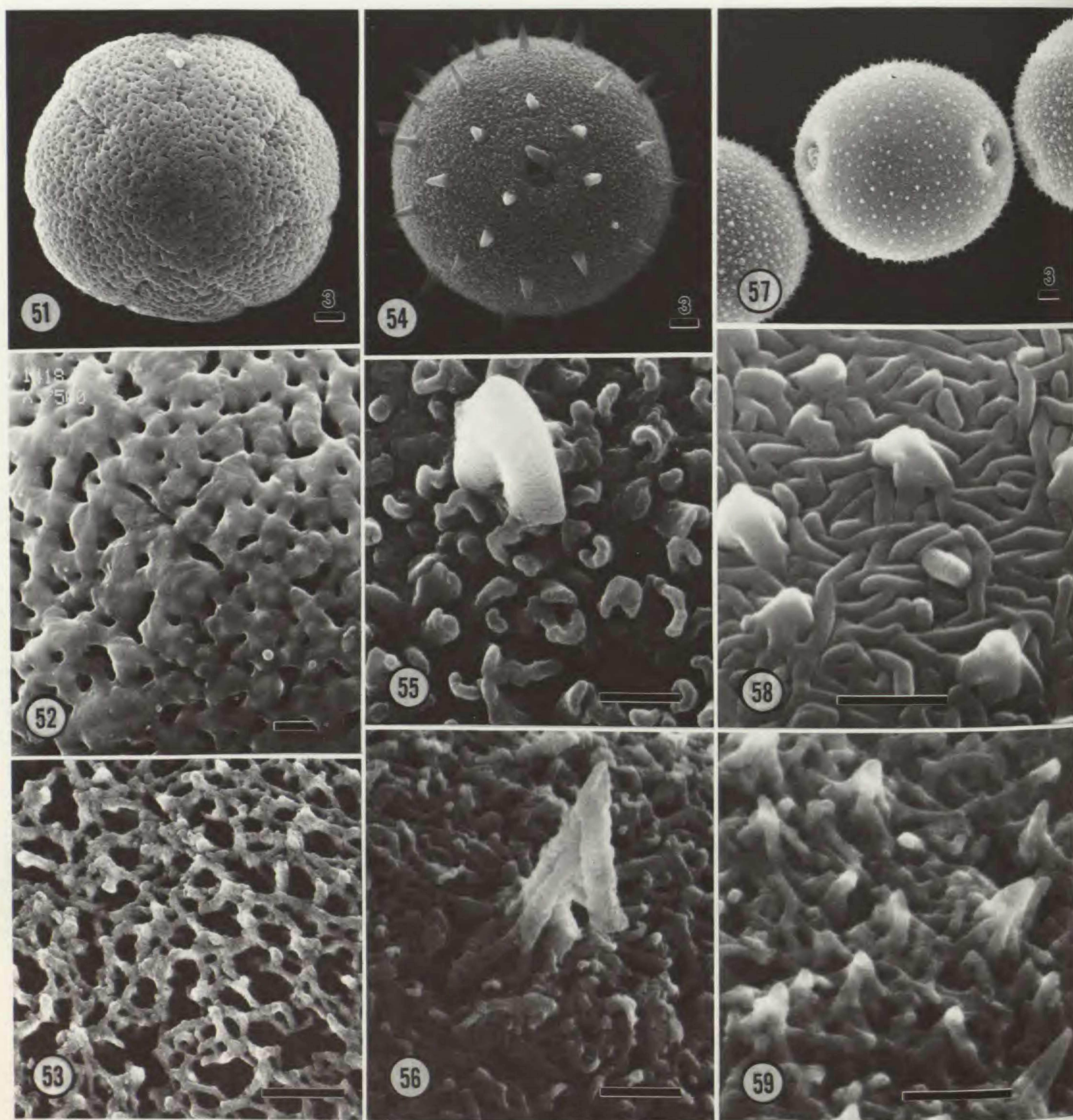
FIGURES 51–59.   SEMs of control and plasma-ashed exines of *Codonopsis* and *Campanula*. 51–53. *Codonopsis bulleyana.* —51. Control. Slightly oblique polar view showing seven colpi.—52. Control. Tectum.—53. Exine plasma-ashed for 90 minutes. See legend of Figure 59. 54–56. *Campanula medium.* —54. Control. Whole grain.—55. Control. Tectum including large spinule.—56. Exine plasma-ashed for 180 minutes. Note that at least part of spinule is a continuation of rods. 57–59. *Campanula baumgartenii.* —57. Control. Whole grain plus parts of two others.—58. Control. Tectum.—59. Exine plasma-ashed for 60 minutes. Plasma-ashing produces skeletonlike exines; in Figure 56 the very prominent spinule appears to be the result of at least four rods coalescing, but the remainder of the tectum is very similar to that in *Codonopsis bulleyana* in Figure 53. In fact, the fundamental structure of the tectum in Figure 53 is not very different from that in Figure 56. When Figure 58 of the control of *Campanula baumgartenii* is compared with the plasma-ashed tectum in Figure 59, the spinules are clearly the result of coalescing of several rods. Scale bars = 1 μm unless otherwise indicated.

umellae that project through the tectum to form the core of most spinules.

The most obvious explanation, and the easiest to dismiss for various reasons, is that the five pantoporate species are closely related. The difference in habit is conspicuous: *Campanula americana* grows up to 2 m tall and has leaves 7–15 cm long (Gleason, 1952; Shetler, 1958, 1962, 1963; Baskin & Baskin, 1984); the California species are 2–25(–40) cm tall with leaves < 11 mm long, except for *C. californica*, which has leaves up to 25 mm long (Munz & Keck, 1973). Small (1903), in his *Flora of the Southeastern United States*, elevated *C. americana* to generic status as *Cam-*

panulastrum americanum (L.) Small, based on its rotate corolla, recurved style, and seemingly spicate inflorescence versus the campanulate corolla, erect style, and mostly terminal flowers in the remaining campanulas (the inflorescence of *C. americana* is basically cymose, as in many campanulas, but is divaricately branched, appearing spicate, racemose, or paniculate (Shetler, 1958). Subsequent authors have not followed Small's treatment, although Shetler & Matthews (1967) argued at one point for its recognition.

The five pantoporate species do not group naturally by the chromosome numbers that have been reported: *Campanula americana* is $n = 29$ (Gadella, 1964; Shetler & Matthews, in 1964 unpublished count), unique in the genus (Gadella, 1964); *C. exigua*, *C. griffinii*, and *C. sharsmithiae* are $n = 17$ (Morin, 1980); *C. angustiflora* is $n = 15$; and *C. californica* has, apparently, not been counted. In a cytological study of 77 species of *Campanula* (Gadella, 1964), the range of diploid numbers was 16 to 102, with 45 species having $2n = 34$, six with $2n = 20$, and another six with $2n = 68$. The relatively high haploid number of *C. americana* suggests allopolyploidy, as does the robust habit and high number of pollen apertures. Conversely, the more diminutive habit of the California pantoporate species and the lower number of apertures (*C. sharsmithiae* excepted) suggest that their numbers are diploid.

Polyploidy is frequently correlated with larger pollen, but this is not the case in these five campanulas: the pollen of *C. sharsmithiae* and of one collection of *C. exigua* (*Sharsmith & Sharsmith 3358*) is just as large or slightly larger than that of *C. americana*. In fact, all 11 collections of pantoporate species examined (Table 1) have overlapping size measurements.

It is unlikely that the pantoporate aperture type and the prominent columellae are genetically linked—prominent columellae occur in other genera of the Campanuloidae that have equatorial apertures, e.g., *Githopsis pulchella* (Fig. 47).

In Campanulaceae, however, all species reported to have relatively high or elongate columellae also have a relatively high number of apertures. Dunbar (1984) reported high columellae for *Githopsis calycina*, *Cyananthus incanus*, and *Codonopsis clematidea*, which are 6-porate, 9-colpate, and 8-colpate, respectively. Perhaps a greater number of apertures makes the exine more vulnerable to collapse, and selective pressures have resulted in a more rigid wall by means of elongate columellae.

We have arrived at much the same conclusion

that Morin (1980) did: pantoporate apertures (and well-developed columellae) have evolved independently at least twice in *Campanula*. Admittedly, this explanation only begs the next question—why, in a large family, should pantoporate apertures evolve twice in the North American species of one genus and be absent in all the other taxa, insofar as they are known?

That the Campanuloideae are a closely related assemblage of species is suggested by the unusual and complex pollination mechanism involving retractile pollen-collecting hairs that apparently is common to all genera and species (reviewed by Shetler, 1979; see also Carolin, 1960, and Lloyd & Yates, 1982). Figures 60–69 of *C. americana* document the stages in pollination: Figure 60 shows a mature style just before anther dehiscence; Figure 61 illustrates the condition just after dehiscence when the concomitant elongation of the lower part of the style causes hairs to "sweep up" pollen; Figure 62 shows a later stage in which much of the pollen has been removed; Figure 63 is a low magnification SEM of a style with most of hairs already retracted; Figure 64 shows two hairs, one (upper) completely invaginated, another in the last stage of invagination; in Figure 65 a pollen grain is partially in the basal lumen; Figures 66–68 show the style with all hairs invaginated and stigmas still not spread; finally, in Figure 69 the stigmatic lobes have been spread, exposing stigmatic surfaces. The invagination mechanism and this sequence are common to all species examined thus far in SEM, some 30 species from different genera (Shetler, unpublished), and thus would appear to be common throughout the Campanuloideae. The mechanism is not known to occur in the Lobelioideae or any other plant family.

The adaptive value of the collecting hairs and their retractile mechanism, unique in the plant kingdom (Uphof, 1962), has long been a matter of speculation but remains to be explained satisfactorily (see Shetler, 1979, for historical review). Although the function of the collecting hairs as a mechanism for indirectly presenting pollen to pollinators is long established, and various workers have demonstrated a relationship between insect activity and the pace of hair retraction as well as pollen removal, there has not been agreement on the exact nature of the relationship between insect activity on the one hand and hair retraction and pollen removal on the other hand.

The bellflowers (Campanuloideae) are all protandrous, with the pollen being swept from the introrse anthers onto the style by the collecting hairs as the flower bud opens and the style elon-
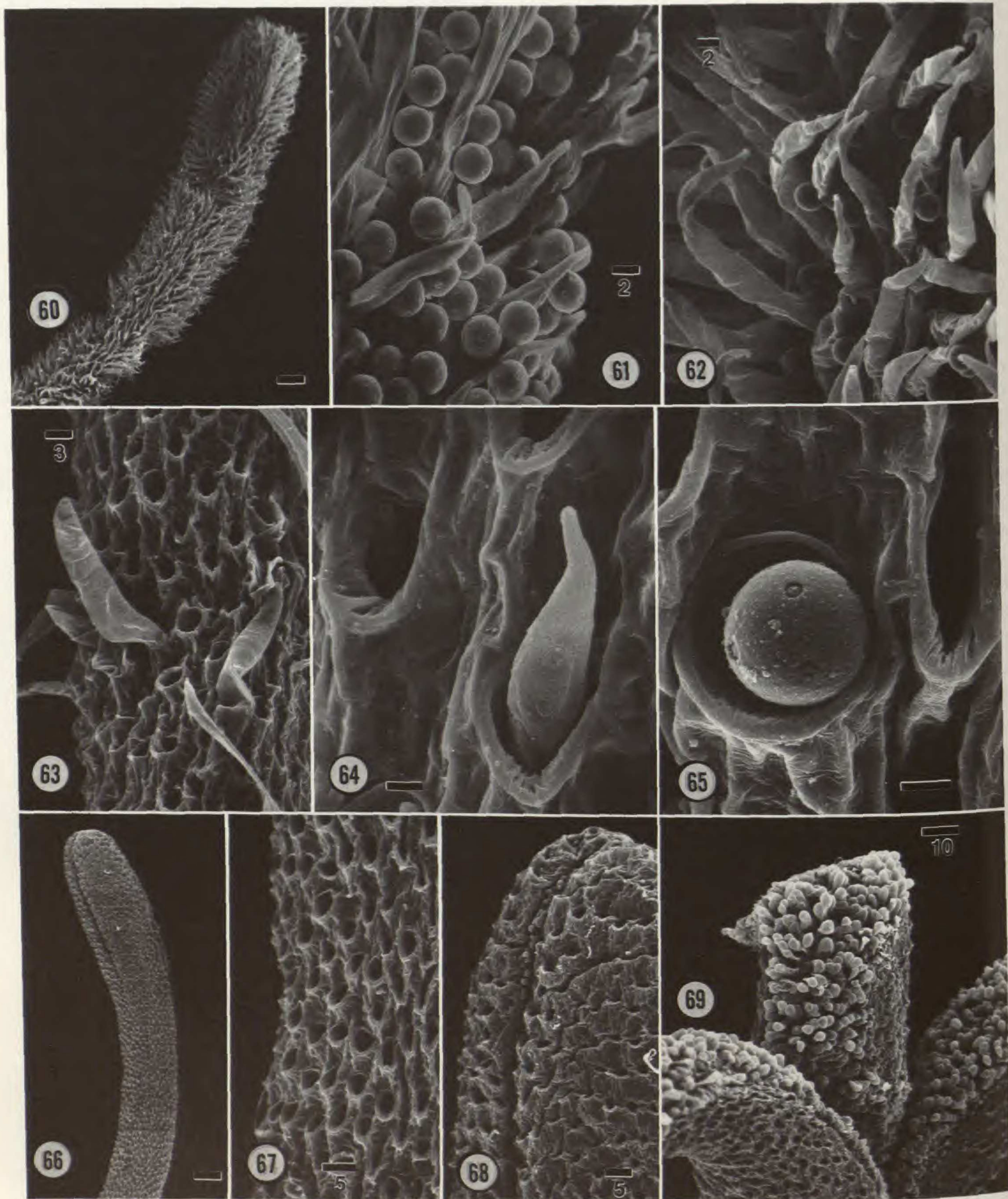
FIGURES 60–69. SEM of pollen collecting hairs of *Campanula americana*. —60. Style with hairs before anthers have dehisced. ×20.—61. Style showing numerous collecting hairs and pollen grains. ×260.—62. Style at later stage, from which most pollen has been removed. ×200.—63. Style at still later stage, in which most of collecting hairs have already invaginated. ×175.—64. Collecting hair in last stage of invagination. ×750.—65. Pollen grain partially invaginated. ×1,000.—66. Style with all hairs invaginated, giving a "pitted" look; stigmas still closed. ×20.—67. See legend of Figure 66. ×110.—68. See legend of Figure 66. ×100.—69. Open stigmas. ×75. Scale bars = 0.5 mm for Figures 60, 66; scale bars = 100 μm for Figures 61–65, 67–69.

gates. When the flower opens the pollen appears as a cylinder around the style reaching to the mouth of the tube or projecting beyond in species with rotate corollas, e.g., *C. americana*. The hairs grad- ually retract from the top of the style downward. In accessing nectar located between the ovary and the expanded filament bases, the pollinators also appear to remove pollen from the top down. Usu-

ally, all hairs are invaginated and all pollen removed before the stigmas spread and expose their surfaces to begin the pistillate phase of the flower.

Kirchner (1897) was the first to suggest that insects could only gather pollen *after* the collecting hairs had retracted and released it. More recently, Carolin (1960) came to the same conclusion, indicating that the pollen could be dislodged by the insects much more easily after the hairs collapsed. Previous observations of *C. americana* and *C. rotundifolia* sens. lat. by Shetler (1958, 1962, 1982) indicate, however, that the hairs retract as the pollen is removed, not before it is removed, and that both hair retraction and pollen removal are slowed when insect activity is absent or limited.

In a study of intrasexual selection and the segregation of pollen and stigma in hermaphroditic plants, Lloyd & Yates (1982) used as their example *Wahlenbergia albomarginata* Hooker, an endemic New Zealand species of this large and wide-ranging genus of Southern Hemisphere bellflowers, which have the typical collecting-hair mechanism. They were able to elucidate more precisely the hair-retracting process, showing that it really is a combination of invagination and telescoping or retracting. They also found that pollen was difficult to remove forcibly, with a fine brush, from the style where the hairs had not yet retracted, but in those cases where it came off easily the hairs proved to have been already retracted. They stated (Lloyd & Yates, 1982: 908) that their observations show that in this species ". . . retraction of the hairs normally precedes the removal of the pollen and therefore supports the hypothesis that retraction facilitates pollen removal."

The evidence presented, however, is not altogether convincing when they say, "The pollen-collecting hairs are usually present among the pollen on those parts of the style which are still abundantly clothed with pollen, but they have retracted into the style, on those parts of the style from which pollen has been removed." This begs the question of which disappears first. They then cite their figure 2, where they explain away an SEM micrograph (fig. 2c) that shows extended hairs without pollen present, as having lost the pollen in preparation. They conclude (Lloyd & Yates 1982: 908) that the collecting hairs function only to collect pollen and are actually detrimental to pollen removal by insects and that "the progressive retraction of the hairs down the style allows pollen to be gradually released from one flower in small increments throughout the male phase." In evolutionary terms, they speculate that this gradual-release mechanism and a prolonged male phase,

which "have the effect of spreading the presentation of pollen in time" and increasing paternal fitness, may be brought about through intrasexual selection.

Richardson & Stephenson (1989), in their recent, controlled observations and experiments with *Campanula rapunculoides* L., found that the "duration of the staminate phase was related to the rate of pollen removal" (p. 535). In general, their findings with respect to the relative duration of the staminate and pistillate phases were consistent with Shetler's findings for *C. americana*. They concur with Lloyd and Yates, however, on the difficulty of removing pollen before the hairs have invaginated and also with their explanation of the gradual retraction of hairs from the stigmatic end downward as a means of reducing the amount of pollen that can be removed in a single insect visit, thus constituting a mechanism for releasing the pollen load incrementally.

Whether the conclusions and hypotheses of Lloyd & Yates (1982) are directly applicable to *Campanula americana* remains unanswered, but one aspect that neither they nor Richardson & Stephenson (1989) mention is the trapping of pollen grains in the basal cavities left by the invaginated hairs (Fig. 65; and Shetler, 1982: fig. 8). This phenomenon has been documented by Shetler (1979, 1982, and unpublished data) for many bellflower species.

We regard the question of the dependence of pollen removal on hair invagination still open and unresolved. Until the actual triggering mechanism (for hair invagination) is understood, the precise relationship of hair invagination to pollen removal will remain debatable. Preliminary unpublished studies by Shetler of *Campanula rotundifolia* show that pollen is removed while collecting hairs are still fully extended, and that individual pollen grains can be "pulled into" the large basal lumina of retracted hairs. (Styles at three stages of maturation from field collections preserved in FAA were embedded in paraffin and thick-sectioned.) Furthermore, Shetler's (1958, 1962, 1982) observations of *C. americana* and *C. rotundifolia* that collecting hairs disappear more slowly when there is little or no insect activity to remove pollen require explanation, and it would appear that Richardson and Stephenson have made similar observations of *C. rapunculoides*.

The palynological data presented here confirm the rarity of the pantoporate aperture type in Campanulaceae, i.e., 34 of the original total of 39 species examined had apertures placed on the equator of the grain. The distinction between panto-

porate and zonoporate aperture types is supported by differences in exine structure: well-developed, projecting columellae in the former versus reduced or short columellae and a thicker foot layer in the latter. The finely perforated tectum and very large columellae of *C. americana* appear to be unique in the genus, if not in the family, and, along with its unique chromosome number ($n = 29$) and morphology, emphasize the distinctiveness of this species. The pantoporate pollen of *C. californica*, in which neither the distribution nor size of the pores is uniform, suggests a transition stage between panto- and zonoporate types. The zonoporate annual *C. angustiflora* is clearly allied to the pantoporate *C. sharsmithiae* in tectum sculpture and exine structure. The irregular distribution and higher number of pores in the former species could also be interpreted as a transition stage between the pantoporate and zonoporate conditions.

ABOUT THE FIGURES

The collections illustrated are given in the Figure(s) column in Table 1. Scales = 1 micron unless indicated otherwise. A number above the scale bar means the length of the bar must be divided by that number in calculating the magnification. For example, in Figure 1, the actual magnification is ×1,200, but it is impossible to cut accurately a bar 1.2 mm long; so a bar was cut 6 mm long and the factor 5 placed above: 6 mm (or 6,000 microns)/5 = 1,200.

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